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Penicillium mallochii and *P. guanacastense*, two new species isolated from Costa Rican caterpillars

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Abstract — Twenty-five strains of monoverticillate *Penicillium* species were isolated from dissected guts and fecal pellets of leaf-eating caterpillars reared in the Área de Conservación Guanacaste, Costa Rica, or from washed leaves of their food plants. Phylogenetic analyses of β-tubulin, nuclear ribosomal internal transcribed spacer (ITS), cytochrome c oxidase subunit 1, translation elongation factor 1-*α* and calmodulin gene sequences revealed two phylogenetically distinct, undescribed species closely related to *P. sclerotiorum*. *Penicillium mallochii* was isolated from *Rothschildia lebeau* and *Citheronia lobesis* (*Saturniidae*) and their food plant *Spondias mombin* (*Anacardiaceae*) and *P. guanacastense* from *Eutelia* sp. (*Noctuidae*). Both fungi produce greenish conidial masses and orange pigments in agar culture, have smooth-walled, monoverticillate conidiophores with moderately vesiculate apices, and globose to subglobose conidia. The species morphologically resemble *P. sclerotiorum* but differ subtly in vesicle width and conidial shape.

Key words — *Penicillium* subg. *Aspergilloides,* sect. *Sclerotiora*, DNA barcoding, All Taxa Biological Inventory (ATBI)

Introduction

In 2001, a microbial observatory was established in Sector Santa Rosa of the UNESCO World Heritage Site, Área de Conservación Guanacaste, northwestern Costa Rica, to culture microbiota (including fungi) associated with caterpillars reared on the tree *Spondias mombin* as a model for elucidating ecological roles of insects and associated microbes in a tropical forest ecosystem.

The fungi isolated from dissected caterpillar guts and fecal pellets were the subject of an MSc thesis (Urb 2006) and included many *Penicillium* strains that required detailed taxonomic study to identify. Several strains were identified as the common and widespread biverticillate *Penicillium citrinum* Thom, but a significant number had unbranched, monoverticillate conidiophores and colonies reminiscent of *P. sclerotiorum* J.F.H. Beyma (Pitt 1979).

As part of the Canadian Barcode of Life Network, we explored DNA barcoding of *Penicillium* species using cytochrome oxidase 1 (cox1; Seifert et al. 2007) and the proposed fungal DNA barcode, the internal transcribed spacer (ITS) of the nuclear ribosomal DNA. In this paper, two new species, *P. mallochii* and *P. guanacastense* are formally described from the Área de Conservación Guanacaste, as part of a broader revision of the *P. sclerotiorum* complex (Rivera & Seifert 2011). The fungi were characterized using cultural and microscopic characters, DNA barcode data, and additional DNA sequence data from β-tubulin (benA), translation elongation factor 1-α (tef1-*α*), and calmodulin (cmd) genes.

Materials & methods

Isolates

Caterpillars of *Rothschildia lebeau* and *Citheronia lobesis* (*Saturniidae*) feeding on *Spondias mombin* (*Anacardiaceae*), *Cochlospermum vitifolium* (*Bixaceae*), or *Exostema mexicanum* (*Rubiaceae*) were collected by Joel Díaz, Felipe Chavarría-Díaz, and Maria Garcia in the tropical dry forest of Sector Santa Rosa of Área de Conservación Guanacaste, northwestern Costa Rica. Caterpillars were surface sterilized with dH_2O and ethanol and guts were removed aseptically and, when possible, partitioned into foregut plus midgut and hindgut sections. Gut contents or feces (obtained from caterpillars kept in sterile Petri dishes) were suspended in sterile H_2O and serially diluted in sterile dH_2O, then plated on soil extract-rose bengal agar (Bills & Foster 2004). Fungal colonies were transferred to Malt Extract Agar (MEA, Samson et al. 2004) until pure cultures were obtained. Cultures are preserved in the Canadian Collection of Fungal Cultures (CCFC, Table 1) and ex-types are also deposited in the CBS-KNAW Fungal Biodiversity Centre culture collection (CBS), Utrecht, the Netherlands. Cultures lacking DAOM numbers are preserved in the personal collection of K. Seifert (KAS) or R.G. Thorn (RGTHC). Details for cultures of other species are given by Rivera & Seifert (2011).

BLAST searches of ITS and benA sequences suggested that the new species were related to *Penicillium sclerotiorum*. When possible, we obtained five strains per species of this clade (Peterson 2000) for comparison, including *P. sclerotiorum*, *P. adametzii* K.M. Zalessky, *P. bilaiae* Chalab., *P. herquei* Bainier & Sartory and *P. hirayamae* Udagawa. Strains were obtained from CBS and the USDA-ARS, National Center for Agricultural Utilization Research culture collection (NRRL).

Morphology

For standardized descriptions, spore suspensions in 0.1% semi-solid agar were inoculated at three points on Czapek Yeast Agar (CYA, Pitt 1979) with added micro-

nutrients (Samson et al. 2004), and MEA in 9 cm polystyrene Petri dishes, and incubated at 25 °C for 7 d in the dark (Samson & Pitt 1985). To determine whether sclerotia could be induced, some strains were incubated for up to 6 months in incident light at room temperature on Oatmeal Agar (OA, Samson et al. 2004). Colony colors were determined using Kornerup & Wanscher (1978); alphanumeric color codes refer to this work. Colony photographs were taken with a Nikon CoolPix E 5000 camera with incandescent lighting and a copy stand.

Microscopic examinations employed an Olympus BX 50 light microscope on material removed from MEA colonies and mounted in 85% lactic acid. Microphotographs were taken with an Evolution MP Camera and microscopic characters were measured from digital images using Image-Pro Plus v. 6 (Media Cybernetics, MD, U.S.A.). For each strain, ten phialides, stipes, metulae and vesicles were measured manually, and 25 automated measurements were made for conidia.

DNA isolation, PCR amplification, DNA sequencing and phylogenetic analyses

Detailed protocols are provided in the revision of the *P. sclerotiorum* complex by Rivera & Seifert (2011), along with details of PCR and sequencing of some strains, and only a summary of relevant details is provided here. DNA was extracted from agar cultures using UltraClean™ Microbial DNA Isolation Kits (MO BIO Laboratories, Inc., Montreal, Canada) following the manufacturer's protocol. Genes were amplified by PCR and sequenced with the following primers: BENA Bt2a and Bt2b (Glass & Donaldson 1995); ITS ITS1 and ITS4 (White et al. 1990); COX1 PF and AR (Seifert et al. 2007); TEF1-α EF1c and EF6 (Peterson et al. 2004); CMD CMD5 and CMD6 (Hong et al. 2006).

PCR reactions were performed in 10 µl reaction mixtures with 1 µl genomic DNA, 1X PCR Buffer, 0.1 mm of dNTPs, 0.08 µM of each primer, and 0.5X Taq polymerase, in a TGradient (Biometra, Montreal, Canada) or a Genius (Techne, Duxford, Cambridge) thermocycler. Sequencing reactions were performed directly from PCR products in both directions, with templates precipitated by ethanol/EDTA/sodium acetate precipitation. Sequences were determined on an ABI 3130XL Genetic Analyzer (Applied Biosystems, Foster City, California, USA).

Consensus sequences were assembled using Sequencher v. 4.8 (Genes Codes Corporation, MI, USA). Alignments were constructed using the online version of MAFFT v. 6 (Katoh et al. 2009), and adjusted to optimize homology using BioEdit 7.0.9 (Hall 1999). Maximum parsimony (MP) analyses were performed using heuristic searches in PAUP v. 4 (Swofford 2002) with the tree bisection-reconnection (TBR) branchswapping algorithm. Maximum likelihood (ML) analyses were performed using Phylogenies for Maximum Likelihood (PhyML) v. 2.4.4 (Guindon et al. 2010). Tree searches for each alignment were run under the nucleotide substitution model obtained from ModelTest 3.7, using the Akaike Information Criteria (AIC) (Posada and Crandall, 1998), and starting from a tree obtained for branch swapping using the modified neighbor joining algorithm BIONJ (Gascuel 1997) as implemented by PhyML. Bayesian inference (BI) analyses were performed using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001), under the same models selected for ML. All Bayesian analyses were performed using random starting trees, and for each gene, four chains were run for one million generations, sampling every 100 generations, generating 10,001 trees, with the first 2,500 discarded as 'burnin' for each chain.

Table 1. *Penicillium mallochii* and *P. guanacastense* strains used in this study. All originate in Área de Conservación Guanacaste, Santa Rosa, Costa Rica.

Penicillium species	Accession number	ACG number	Caterpillar	Food Plant	GenBank Accession numbers				
					ITS	benA	tef-1α	cmd	cox1
mallochii	DAOM 239917*	03-RGTHC-903	none	*S. mombin*	JN626104	JN625973	JN626152	JN626016	JN626065
	DAOM 239918	01-RGTHC-144	*Rothschildia lebeau* (entire gut)	*S. mombin*	JN626105	JN625974	JN626153	JN626017	JN626066
	DAOM 239919	02-RGTHC-474	*Citheronia lobesis* (MG)	*S. mombin*	JN626106	JN625975	JN626154	JN626018	JN626067
	DAOM 239920	03-RGTHC-895	*R. lebeau* (HG)	*S. mombin*	JN626107	JN625976	JN626155	JN626019	JN626068
	DAOM 239921	03-RGTHC-900	*R. lebeau* (FG, MG)	*S. mombin*	JN626108	JN625977	JN626156	JN626020	JN626069
	DAOM 239922	02-RGTHC-386	*R. lebeau* (frass)	*S. mombin* [1]	JN626109	JN625978	JN626157	JN626021	JN626070
	DAOM 239924	02-RGTHC-607	*C. lobesis* (FG, MG)	*C. vitifolium*	JN626110	JN625979	JN626158	JN626022	JN626071
	DAOM 239925	02-RGTHC-599	*C. lobesis* (FG, MG)	*C. vitifolium*	JN626112	JN625980	JN626159	JN626023	JN626072
	DAOM 239926	02-RGTHC-383	*R. lebeau* (frass)	*S. mombin*	JN626111	JN625981	JN626160	JN626024	JN626073
	DAOM 239927	01-RGTHC-311	*R. lebeau* (entire gut)	*S. mombin*	JN626113	JN625982	JN626161	JN626025	JN626074
	DAOM 239928	02-RGTHC-676	*C. lobesis* (FG, MG)	*C. vitifolium*	JN626114	JN625983	JN626162	JN626026	JN626075
	DAOM 239929	03-RGTHC-893	*R. lebeau* (HG)	*S. mombin*	JN626115	JN625984	JN626163	JN626027	JN626076
	KAS 2597	01-RGTHC-294	*R. lebeau* (entire gut)	*S. mombin*	JN626116	JN625985	JN626164	JN626028	JN626077
	KAS 2601	02-RGTHC-481	*C. lobesis* (MG)	*S. mombin*	JN626117	JN625986	JN626165	JN626029	JN626078
	KAS 2606	02-RGTHC-679	*C. lobesis* (FG, MG)	*C. vitifolium*	JN626118	JN625987	JN626166	JN626030	JN626079

Penicillium species	Accession number	ACG number	Caterpillar	Food Plant	GenBank Accession numbers				
					ITS	benA	tef-1α	cmd	cox1
mallochii (continued)	kas 2612	03-rgthc-911	none	*S. mombin*	JN626119	JN625988	JN626167	JN626031	JN626080
	kas 2615	02-rgthc-387	*R. lebeau* (frass)	*S. mombin*	JN626120	JN625989	JN626168	JN626032	JN626081
	kas 2630	02-rgthc-389	*R. lebeau* (frass)	*S. mombin*	JN626121	JN625990	JN626169	JN626033	JN626082
	kas 3183	03-rgthc-891	*R. lebeau* (hg)	*S. mombin*	JN626122	JN625991	JN626170	JN626034	JN626083
	kas 3186	03-rgthc-770	*C. lobesis* (entire gut)	*S. mombin*	JN626123	JN625992	JN626171	JN626035	JN626084
	kas 3187	03-rgthc-894	*R. lebeau* (hg)	*S. mombin*	JN626124	JN625993	JN626172	JN626036	JN626085
	kas 3188	02-rgthc-621	*R. lebeau* (fg, mg)	*E. mexicanum*	JN626125	JN625994	JN626173	JN626037	JN626086
	kas 3190	02-rgthc-627	*R. lebeau* (fg, mg)	*E. mexicanum*	JN626126	JN625995	JN626174	JN626038	JN626087
	kas 3191	03-rgthc-896	*R. lebeau* (fg, mg)	*S. mombin*	JN626127	JN625996	JN626175	JN626039	JN626088
	kas 3193	03-rgthc-890	*R. lebeau* (hg)	*S. mombin*	JN626128	JN625997	JN626176	JN626040	JN626089
guanacastense	daom 239912*	02-rgthc-462	*Eutelia* sp. (entire gut)	*S. mombin*	JN626098	JN625967	JN626146	JN626010	JN626059
	daom 239913	02-rgthc-317	*Eutelia* sp. (entire gut)	*S. mombin*	JN626099	JN625968	JN626147	JN626011	JN626060

* Abbreviations: daom = Canadian Collection of Fungal Cultures; kas = personal culture collection of Keith Seifert; rgthc = accession numbers associated with the Guanacaste ATBI; fg = foregut; mg = midgut; hg = hindgut; * = ex-type strain. [1] *S = Spondias; C. = Cochlospermum; E. = Exostema*

All sequences for the two new species are deposited in GenBank with the accession numbers JN625955–JN626181 (Table 1). Details of strains of other species, and the GenBank accession numbers for their sequences, are provided by Rivera & Seifert (2011).

Results

Representative ML trees for each of the five genes (ITS, *cox1,* benA, cmd and tef1-*α*) are presented in Fig. 1. The MP and BI trees are not shown but differences in topology are discussed, and support values for each analysis are mapped onto the ML trees and included in Table 2. A combined gene tree is included in the revision of the entire *P. sclerotiorum* complex (Rivera & Seifert 2011).

Table 2. Statistical support for the monophyletic clades representing *Penicillium mallochii* and *P. guanacastense* in five gene data alignments. *

		P. mallochii	*P. guanacastense*
ITS	MP bootstrap (%)	99	100
	ML probability	0.998	1.00
	BI posterior probability	1.00	1.00
cox1	MP bootstrap (%)	85	100
	ML probability	0.986	0.996
	BI posterior probability	0.53	1.00
tef1-α	MP bootstrap (%)	91	100
	ML probability	0.975	1.00
	BI posterior probability	0.87	1.00
benA	MP bootstrap (%)	100	100
	ML probability	0.998	1.00
	BI posterior probability	1.00	1.00
cmd	MP bootstrap (%)	100	100
	ML probability	1.00	1.00
	BI posterior probability	1.00	1.00

*analyzed by Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference.

Both *P. mallochii* and *P. guanacastense* received strong support as distinct monophyletic clades in all analyses of all five genes (Table 2). The MP benA trees revealed two groups of strains within *P. mallochii* 86% and 70% bootstrap support, which are absent in the other gene trees. These groups had 0.866 and 0.814 ML probabilities, and BI posterior probabilities of 0.74 and 0.66. Long branch lengths subtending these two clades on the MP tree appear to be an artifact, sometimes experienced with maximum parsimony analyses.

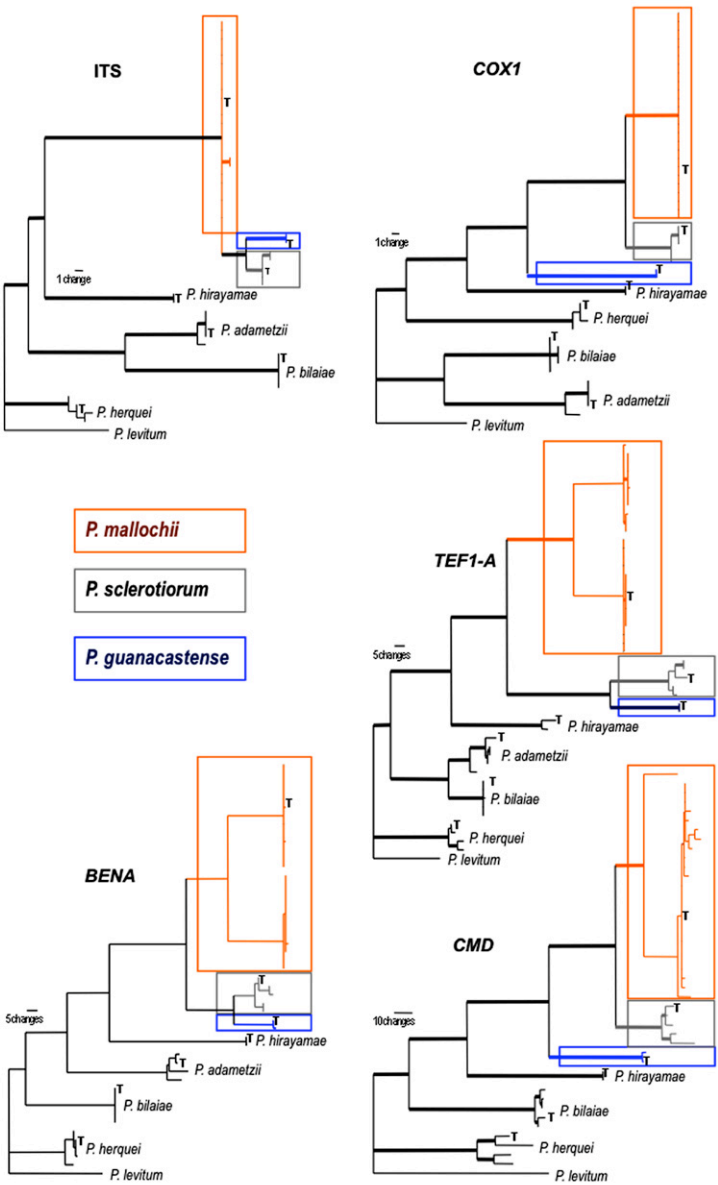


Fig 1. Maximum likelihood (ML) gene trees for ITS, cox1, tef1-α, benA and cmd data sets. The negative-log likelihood (–lnL) of the ML trees are –1497.83287, –1610.84725, –3299.83157, –2712.50962, –3454.53854. Branches with ML-bootstrap support >95%, MP-bootstrap support >95%, and Bayesian Inference partition probabilities >0.99 are thickened.

Taxonomy

Penicillium mallochii K.G. Rivera, Urb & Seifert**, sp. nov.** Figs 2, 4–8

MycoBank MB 563043

Growth on CYA 29–39 mm, on MEA 24–35 mm diam. after 7 days. Conidiophores monoverticillate, stipes 50–380 × 2–4 µm, terminal vesicle 4–6 µm wide, rarely with a single metula 15–45 × 1.5–2 µm. Phialides 7–10(16) × 2–3(4) µm. Conidia (sub)globose, 2.5–3.5 × 2–2.5 µm.

Type: Costa Rica, Santa Rosa, Área de Conservación Guanacaste, isolated from leaf of *Spondias mombin*, 2003, leg. Joel Díaz, Felipe Chavarría-Díaz, and Maria Garcia no. 03-RGTHC-903, isol. M. Urb (**Holotype**, DAOM 239917, dried culture on Blakeslee's MEA; ex-type culture, CCFC 239917).

Etymology: '*mallochii*' named for Prof. David Malloch, retired mycologist from the University of Toronto, now a visiting fellow at the Natural History Museum in St. John, New Brunswick, an expert on ascomycetes, and a mentor to KGR, RGT and KAS.

Colonies on CYA after 7 days at 25°C: typically (24–)29–39 mm diam.; dense and velutinous, sometimes with aerial mycelium in the center; moderately sulcate, with 4–7 grooves, often wrinkled in the center; approximately 1 mm deep; conidia Turquoise Grey to Greenish Grey (24–25D2–3), concentric rings of different shades of these colors present; clear yellow exudate droplets produced moderately in some strains, absent in others; vivid orange soluble pigment present in some strains, absent in others; 2–3 mm of white mycelia at the margin, margin entire; reverse deep Yellow (4A8), Reddish Orange (4A6–7) or Golden Yellow to Orange (5–6A–B6–8) in the center, Light to Pastel Yellow (3–4A4–5) at the margins.

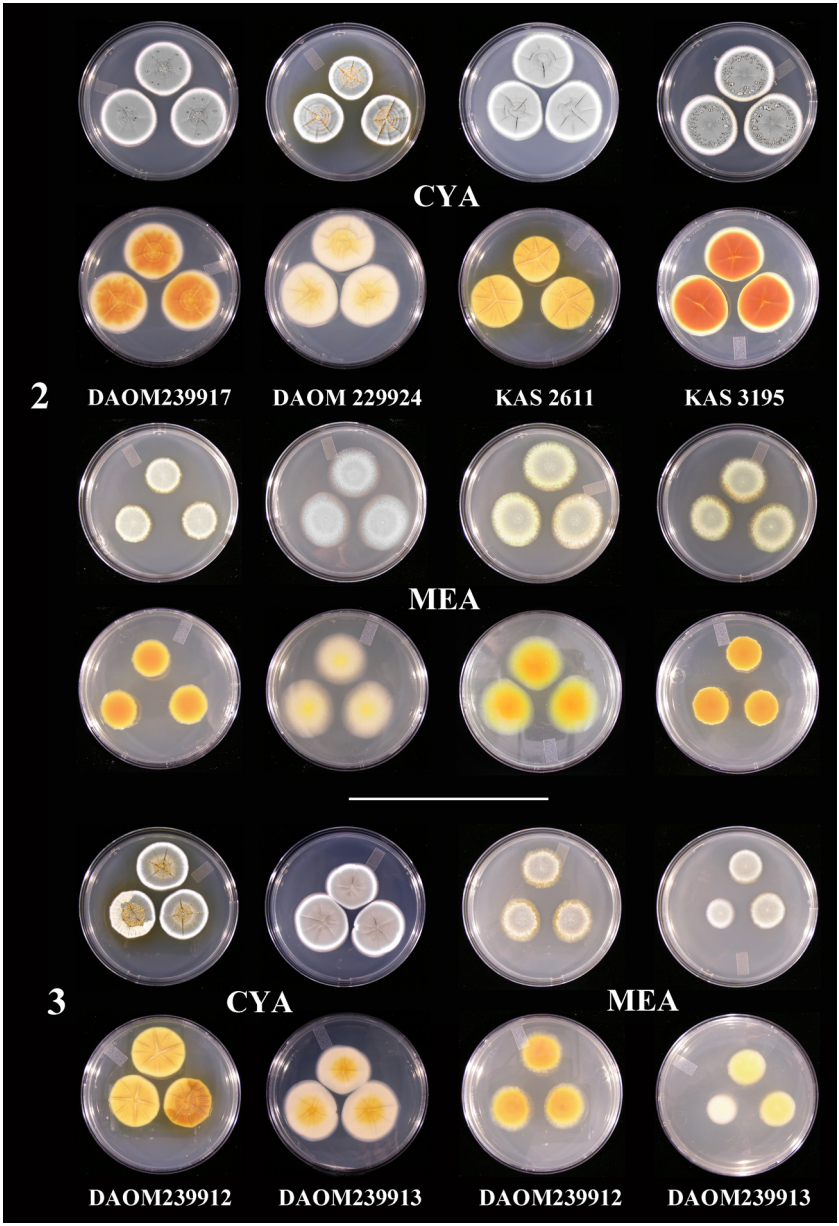
Colonies on MEA after 7 days at 25°C: (19–)24–35 mm diam; planar, velutinous, with no aerial mycelium; conidia Greyish Green to Dull Green (25–30B–E3–7), sometimes forming crusts that dislodge when the colony is disturbed, no exudates or soluble pigments present; reverse Greyish Green (30C5), Greyish Yellow (1–2B–C3–8), Yellow (2–3A6–7), Deep Yellow (4A8) or Orange (6A–B6–8), near the margins paler shades of these colors; margin entire.

Conidiophores monoverticillate, borne from agar surface, stipes septate, 53–380 × 2–4 µm, smooth to finely roughened; mostly unbranched, but in some strains ~10% of the conidiophores have single branches 15–45 × 1.5–2 µm; vesicles 4–6 µm diam (mean for different strains = 4.5–6.0 ± 0.71 µm, n = 25). Phialides 7–10(–16) × 2–3(– 4) µm, ampulliform with a collula. Conidia globose to subglobose, finely roughened, 2.5–3.5 × 2–2.5 µm (range of means for different strains = 2.6–3.4 ± 0.01 × 2.3–2.4 ± 0.01, n = 25), mean L/W ratio 1.15:1.

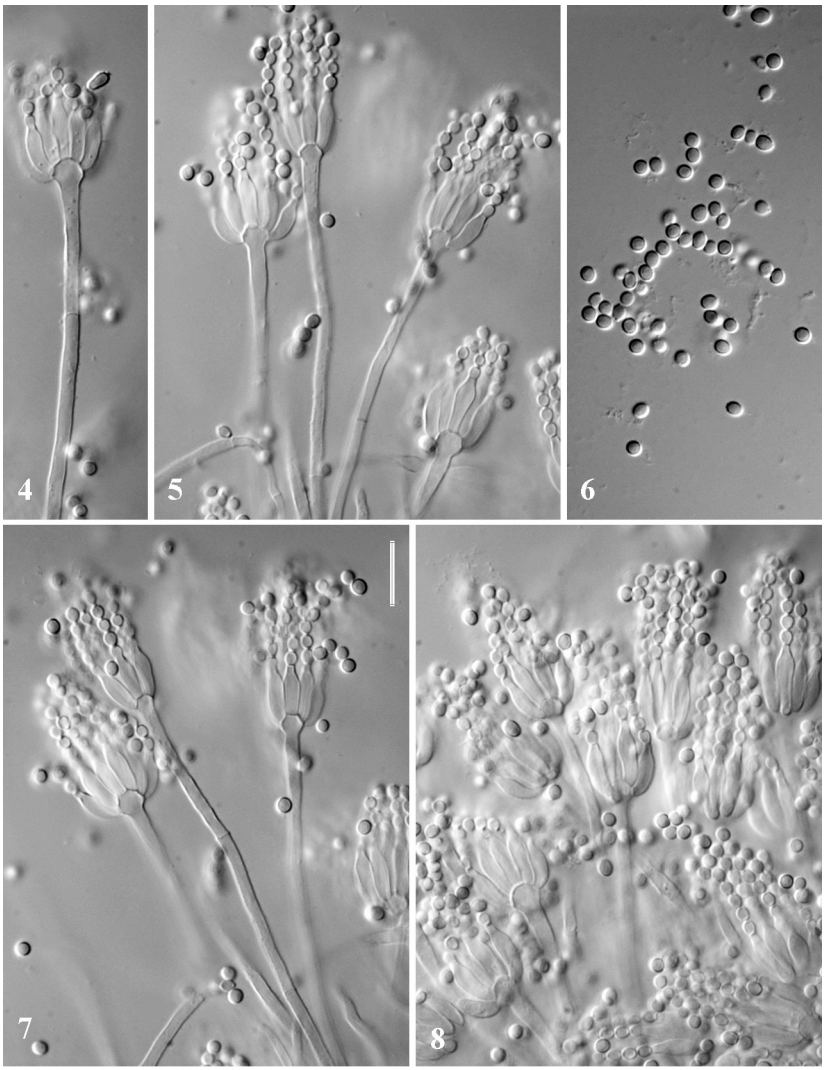
Distribution: Área de Conservación Guanacaste, Sector Santa Rosa, Guanacaste Province, northwestern Costa Rica.

Habitat: *Rothschildia lebeau* and *Citheronia lobesis* (*Saturniidae*) caterpillar guts and faeces, and leaves of *Spondias mombin* (*Anacardiaceae*).

Additional material examined: See Table 1.



Figs 2–3. Seven day old cultures of *Penicillium mallochii* (2) and *P. guanacastense* (3) on CYA and MEA, showing variation in colony characters.



Figs 4–8. *Penicillium mallochii,* photomicrographs from the holotype. 4–5 and 7–8. Monoverticillate conidiophores. 6. Conidia. Scale bar = 10 µm.

Penicillium guanacastense K.G. Rivera, Urb & Seifert**, sp. nov.** Figs 2, 9–12

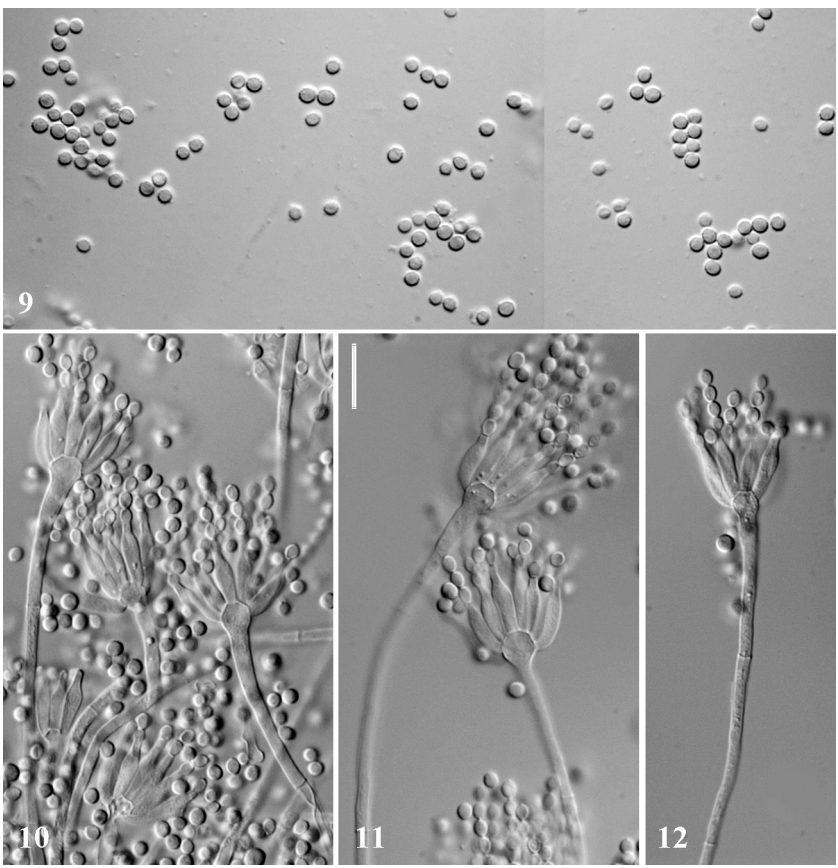
MycoBank MB 563044

Growth on CYA 25–33 mm, on MEA 29–33 mm diam. after 7 days. Conidiophore monoverticillate or rarely with one metula, stipes 85–100 × 2.5–3 µm, terminal vesicle 4–5.5 µm wide. Phialides 8.5–12 × 2–3.5 µm. Conidia globose, 2.5–3.0 µm diam.

Type: Costa Rica, Santa Rosa, Área de Conservación Guanacaste, isolated from *Eutelia* sp. reared on leaf of *Spondias mombin*, 2002, leg. Joel Díaz, Felipe Chavarría-Díaz, and Maria Garcia no. 02-RGTHC-462, isol. M. Urb (**Holotype**, DAOM 239912, dried culture on Blakeslee's MEA; ex-type culture, CCFC 239912).

Etymology: '*guanacastense*' named for the Área de Conservación Guanacaste, where the fungus was discovered.

Colonies on CYA after 7 days at 25°C: 25–33 diam, dense, velutinous, sulcate, with 5–8 grooves, sometimes with sectors with poor sporulation; conidia Greenish Grey (25–30B–E2), with yellow concentric ring near the margins and a bluish grey concentric ring towards the center, clear exudate droplets produced moderately and yellow soluble pigments produced by one strain; 2–3



Figs 9–12. *Penicillium guanacastense,* photomicrographs from the holotype. 9. Conidia. 10–12. Monoverticillate conidiophores. Scale bar = 10 μm.

mm of white mycelium at the margin, margin entire; reverse Orange Yellow to Orange (4–6B7–8).

Colonies on MEA after 7 days at 25°C: 29–33 mm diam., planar, velutinous, no aerial mycelia observed; conidia Greenish Grey (25–30B–E2), sometimes forming crusts that dislodge when the colony is disturbed, with no exudates or soluble pigments present; reverse Yellowish White (2–4B2), Orange (6B7–8) or Light Brown (6–7D4–8), with paler shades of these colors near the margin.

Conidiophores monoverticillate on MEA, borne directly from agar surface; stipes finely roughed, septate 85–100 × 2.5–3 µm; unbranched or sometimes up to ~10% have single branches 15–21 × 2.5–3 µm; vesicles 4–5.5 µm (mean 4.5–4.65 ± 0.5 µm). Phialides 8.5–12 × 2–3.5 µm, ampulliform with distinguishable collula. Conidia globose, finely roughened, 2.5–3 µm diam (range of means for different strains 2.7–2.8 ± 0.01 × 2.5–2.6 ± 0.01 µm, n=25), mean L/W ratio 1.1:1.

Distribution: Área de Conservación Guanacaste, Sector Santa Rosa, Guanacaste Province, northwestern Costa Rica.

Habitat: Guts of *Eutelia* caterpillar (*Noctuidae*) feeding on foliage of *Spondias mombin* (*Anacardiaceae*).

Additional material examined: See Table 1.

Discussion

Penicillium mallochii and *P. guanacastense* are phylogenetically related and morphologically very similar to *P. sclerotiorum*. All three species have vesiculate, monoverticillate conidiophores and vivid orange to red colony reverses on CYA. Morphological characters place both species in *Penicillium* subg. *Aspergilloides* ser. *Glabra* (Pitt 1979), and the recently erected, phylogenetically and morphologically defined sect. *Sclerotiora* (Houbraken & Samson 2011). All species in this series have long, monoverticillate conidiophores with a swollen or vesiculate apex. The conidiophores of *P. sclerotiorum* sensu stricto are conspicuously vesiculate (mean 3.6 ± 0.4 µm), while those of *P. mallochii* and *P. guanacastense* are only moderately vesiculate, but with the vesicles of *P. mallochii* (3.1 ± 0.2 µm) being slightly more swollen than those of *P. guanacastense* (3.0 ± 0.3 µm). Furthermore, *P. sclerotiorum* has ellipsoidal conidia, while *P. mallochii* and *P. guanacastense* have globose to subglobose conidia. Some strains of *P. sclerotiorum* produce pale colored sclerotia (Pitt 1979), which were not observed in any of our *P. mallochii* and *P. guanacastense* strains.

There are ecological differences among these three species. *Penicillium mallochii* strains were isolated from saturniid caterpillars and leaves of their food plant *Spondias mombin*, whereas *P. guanacastense* was isolated from noctuid caterpillars, also feeding on *Spondias mombin*. In contrast, *P. sclerotiorum* strains have mostly been isolated from the soil and the air.

Based on our five-gene analysis of multiple strains of each species, *P. mallochii, P. guanacastense,* and *P. sclerotiorum* are phylogenetically distinct species. Each species was resolved as a strongly supported monophyletic group with each gene, with each kind of analysis, except for the ITS MP and BI analyses, which did not resolve *P. mallochii* from the branch that grouped *P. guanacastense* and *P. sclerotiorum*.

In the study of microfungi isolated from guts and frass of two species of caterpillars feeding on the tree *Spondias mombin*, *Penicillium* was the fourth most commonly isolated of the 48 identified genera, comprising 88 of 835 isolates (Urb 2006). Twenty-four strains of *P. citrinum* were isolated, and this was the most frequent species isolated from guts of *Citheronia lobesis* (with three β-tubulin variants, represented by GenBank accession numbers JN637993–JN637995). A similar number of strains of *P. mallochii* were isolated from *Rothschildia lebeau* (Urb 2006). Whether these species are phylloplane inhabitants or endophytes of *S. mombin* leaves is unknown, and the significance of their frequent association with these caterpillars remains a subject for future research.

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